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A Prospective Study of Marine Phytoplankton and Reported Illness among Recreational Beachgoers in Puerto Rico, 2009

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Abstract

Background: Blooms of marine phytoplankton may adversely affect human health. The potential public health impact of low-level exposures is not well-established, and few prospective cohort studies of recreational exposures to marine phytoplankton have been conducted.

Objective: We evaluated the association between phytoplankton cell counts and subsequent illness among recreational beachgoers.

Methods: We recruited beachgoers at Boquerón Beach, Puerto Rico during the summer of 2009. We conducted interviews at three time points to assess baseline health, water activities, and subsequent illness. Daily water samples were quantitatively assayed for phytoplankton cell count. Logistic regression models, adjusted for age and sex, were used to assess the association between exposure to three categories of phytoplankton concentration and subsequent illness.

Results: During 26 study days, 15,726 individuals successfully completed all three interviews. Daily total phytoplankton cell counts ranged from 346 to 2,012 cells/mL (median: 712 cells/mL). The highest category ($\geq 75^{\text{th}}$ percentile) of total phytoplankton cell count was associated with eye irritation (Adjusted Odds Ratio (OR)=1.30; 95% confidence interval (CI): 1.01-1.66), rash (OR=1.27; 95% CI: 1.02-1.57), and earache (OR=1.25; 95% CI: 0.88, 1.77). In phytoplankton group-specific analyses, the highest category of Cyanobacteria counts was associated with respiratory illness (OR=1.37; 95% CI: 1.12-1.67), rash (OR=1.32; 95% CI: 1.05-1.66), eye irritation (OR=1.25; 95% CI: 0.97-1.62), and earache (OR=1.35; 95% CI: 0.95-1.93).

Conclusions: We found associations between recreational exposure to marine phytoplankton and reports of eye irritation, respiratory illness, and rash. We also found that associations varied by phytoplankton group, with Cyanobacteria having the strongest and most consistent associations.

Introduction

Harmful algal blooms occur when phytoplankton accumulate and negatively impact the environment and human or animal health. Harmful blooms are associated with a small subset of phytoplankton species. Out of 4,000 marine phytoplankton, it is estimated that some 200 are high-biomass producers, and only around 80 are potential toxin-producers (Maso and Garces 2006; Smayda 1997; Smayda and Reynolds 2003; Zingone and Enevoldsen 2000). Although naturally occurring in fresh, estuarine, and marine waters, the growth, toxicity, and geographic distribution of harmful algae have increased with environmental factors such as nutrient enrichment and warmer water temperatures (Dyble et al. 2008; Moore et al. 2008; Paerl et al. 2001; Paerl and Huisman 2008, 2009; Sellner et al. 2003). Most epidemiologic studies of harmful algal blooms, specifically those generated by cyanobacteria, have been conducted at freshwater sites. In the U.S., freshwater harmful algal blooms have been associated with waterborne disease outbreaks that include dermatologic, gastrointestinal, respiratory, febrile, ear, and eye symptoms (Dziuban et al. 2006; Hilborn et al. 2014; Yoder et al. 2004). The World Health Organization (WHO) has established guidelines for cell count categories associated with the risk of human health effects (Bartram and Chorus 1999). The lowest guidance level of 20,000 cyanobacterial cells per ml was derived from an epidemiologic study of freshwater cyanobacteria exposure (Pilotto et al. 1997). Currently, there is no federal regulation of cyanobacteria or cyanotoxin exposure among recreational waters in the U.S.; however, several state and local governments have established guidelines for exposure based on their own or the WHO risk assessments (Burch 2008).

Adverse human health outcomes have been associated with marine dinoflagellates, diatoms, and cyanobacteria (World Health Organization 2003). For example, harmful algal

blooms produced by *Karenia brevis*, a marine dinoflagellate, have been reported to produce brevetoxins that are associated with gastrointestinal and respiratory illness (Backer et al. 2003; Backer et al. 2005; Hlavsa et al. 2011; Kirkpatrick et al. 2010). *Lyngbya majuscula*, a benthic marine cyanobacterium, is known to produce toxins, such as debromoaplysiatoxin and lyngbyatoxin, and acute dermal lesions among swimmers (Nagai et al. 1996; Osborne et al. 2001; Osborne et al. 2007; Osborne and Shaw 2008; World Health Organization 2003). The picoplanktonic *Synechococcus*, a cyanobacterium, has been reported to produce microcystins, a group of potent hepatotoxic cyanotoxins (Carmichael and Li 2006). A limited number of epidemiologic studies have investigated the effects of harmful marine algal exposures and most have focused on blooms of *K. brevis* (Backer et al. 2003; Kirkpatrick et al. 2010). As a result, thresholds or concentrations of phytoplankton associated with adverse health effects are not well-established in marine waters.

Given the association between warmer ocean temperatures and increased frequency of harmful algal blooms around the world, there is a need to better understand the impact on human health as climate change progresses (Dale et al. 2006; Gingold et al. 2014; Moore et al. 2008; Peperzak 2005). The objective of this study was to evaluate the association between phytoplankton cell counts and subsequent illness among recreational beachgoers at a tropical marine beach.

Methods

We conducted a prospective study of beachgoers to Boquerón Beach, Puerto Rico in the summer of 2009. We assessed the relationship between phytoplankton counts and the development of illness after recreational exposure. This study was in the context of the National Epidemiological and Environmental Assessment of Recreational (NEEAR) Water Study. A

description of the study design, objectives, and protocols, and a report of the associations between fecal indicator organisms and swimming-associated illness has been published (Wade et al. 2011).

Site description

Boquerón Beach is located on the southwest coast of Puerto Rico (Figure 1). It is approximately 1 mile long and is located on Boquerón Bay, adjacent to the Caribbean Sea.

Interviews

We offered beachgoers enrollment in the study at the beach on summer weekends and holidays for a total of 26 days. Inclusion criteria consisted of: 1) an adult household member ≥ 21 years of age; 2) completion of three interviews (enrollment, beach exit, follow-up); and 3) no previous participation in the study within the prior 28 days. An adult answered questions for all other household members at the beach. Interviews began at Boquerón Beach on May 16, 2009 and concluded on August 2, 2009.

Participants gave informed consent for study participation in their chosen language, English or Spanish, and were interviewed by study personnel proficient in the language. All study materials were approved by the Institutional Review Board of the University of North Carolina at Chapel Hill. The enrollment interview collected information about demographics, baseline health, chronic health conditions, and contact information for follow-up. The exit interview collected information about water exposure and other activities engaged in at the beach that day. Water exposure was ascertained with the following questions: “did you immerse your body in water today”, “did you put your face in water or submerge head in water today?” and “did you swallow the water?” The telephone interview conducted 10-12 days later, recorded self-reported illness experienced since the beach visit. Participants were offered incentives, such

as a cooler, tote bag, or beach-related item, to encourage completion of the exit interview. A \$25 check was provided to each household after the follow-up telephone interview was completed.

Health Endpoints

The health endpoints were defined *a priori* and were similar to those previously studied in relation to recreational water quality and health (Colford et al. 2007; Pruss 1998; Wade et al. 2010). They included incident cases of gastrointestinal (GI) illness, respiratory illness, rash (independent of sunburn), earache, and eye irritation occurring during the 10-12 day period between the beach visit and the follow-up telephone interview. The definitions for each illness are shown in Table 1. Signs and symptoms of each health endpoint were asked separately. For example, the following was asked for stomachache: “have you had a stomachache or abdominal cramping since the beach interview?” Individual signs/symptoms of illness included: stomachache; diarrhea; nausea; vomiting; urinary tract infection; fever; headache; sore throat; cough; cold; runny or stuffy nose; earache, ear infection, or runny ears; watery eyes; eye infection; infected cut; and rash or itchy skin. For each question, participants could answer yes or no. They also had to option to refuse to answer or say they did not know.

Water Samples

Three fixed transects were selected at least 60 meters apart to encompass the majority of the beach site. Water at sites along each transect was repeatedly sampled for marine phytoplankton counts and toxins; samples were collected from the waist depth at each location at 11:00 AM each study day (Figure 1). All samples were refrigerated or placed on ice within 30 minutes of collection and were maintained under refrigeration during shipment to GreenWater Laboratories/CyanoLab (Palatka, Florida) for analysis. Water samples were combined into a daily composite sample and quantitatively assayed for total and group phytoplankton cell counts

(cells/mL) by an experienced phycologist using the counting method described in *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association 1998). The limit of detection using 5 mL samples at 100x magnification was 0.2 cells/mL. Water samples were also analyzed for two cyanobacterial toxins, lyngbyatoxin-a and debromoaplysiatoxin, using high performance liquid chromatography-mass spectrometry (HPLC-MS) (Nagai et al. 1996; Osborne et al. 2008). These toxins were selected *a priori* based on the characteristics of the site; the limit of detection for both toxins was 1.0 ppb. Major phytoplankton groups were identified and included Cyanobacteria, Dinophyta (dinoflagellates), Bacillariophyta (diatoms), and miscellaneous other groups and morphotypes.

Statistical analysis

Multivariable logistic regression was used to evaluate the association between exposure to different categories of phytoplankton cell count and incidence of reported illness. The outcome was a binary indicator for each health endpoint as defined in Table 1. Each outcome was modeled separately. Each analysis excluded participants who reported having the outcome of interest in the 3 days prior to their beach visit.

Phytoplankton cell count was categorized as high ($\geq 75^{\text{th}}$ percentile), medium ($> 25^{\text{th}}$ to $< 75^{\text{th}}$ percentile), and low ($\leq 25^{\text{th}}$ percentile). The lowest category served as the referent in the regression models. Cell counts were considered in total and by phytoplankton group (e.g., Bacillariophyta, Cyanobacteria, Dinophyta). Picocyanophytes, a subgroup of Cyanobacteria, were examined separately from the Cyanobacteria group because they occurred at an order of magnitude higher than other groups. In order to focus on those with recreational water contact, only participants who reported body immersion were included in models of the association between phytoplankton concentration and illness.

Covariates based on information from previous studies were considered for inclusion in the final model. Using frequency tables and chi-square tests, we identified factors associated with illness and/or water exposure to potential phytoplankton. These factors included age, sex, any other chronic illnesses, self-reported contact with algae, *Enterococcus* count, and digging in sand. After adjusting for different combinations of covariates, the association between phytoplankton exposure and incidence of reported illness varied by less than 0.1. The final model (adjusting for age and sex) was based on minimizing the Akaike information criterion (AIC) in order to balance model parsimony and fit. In sensitivity analyses, we considered other definitions of water exposure: head immersion and swallowing water. Duration of time spent in the water was evaluated as a potential effect measure modifier in stratified analyses.

Results

Respondent characteristics and demographics

During the 26 study days, we included 15,726 individuals from 6,611 households. This represented 76% of the households initially approached and 96% of those completing the beach interview. There were slightly more females (55%) and nearly all (>99%) participants self-identified as Hispanic. The average age was 30 years (range: <1 to 92 years); children under 12 comprised less than 20% of the study population. About a quarter of all participants reported having a chronic illness: 13% reported having allergies, 11% reported having asthma, 5% reported having a chronic GI illness, and 3% reported having a chronic skin condition. Women reported more chronic illnesses, specifically chronic GI illness (6% women; 3% men; χ^2 p-value < 0.0001) and allergies (14% women; 11% men; χ^2 p-value = 0.0004). Age categories (0-4 years; 5-11 years; 12-19 years; 20-34 years; 35-64 years; 65+ years) revealed differences in chronic illness (χ^2 p-value = 0.0002); chronic GI illness increased with age, reports of allergies

were infrequent among the youngest participants (0-4 years), and reports of asthma were most frequent among children less than 12 years of age. Participants were excluded from analyses if they reported having the illness being evaluated in the 3 days before their beach visit. At enrollment, 8% of participants reported having a sore throat, and <3% reported vomiting, other GI illness, rash, eye irritation, or earache in the previous 3 days. Table 2 summarizes the basic characteristics for all study participants and for those who reported body immersion in the water.

Beach Visit Activities

Upon leaving the beach, 77% of all participants reported body immersion in the water, 64% reported head immersion, and 36% reported getting water in their mouth. Table 3 summarizes a sample of activities that participants reported engaging in at the beach. We analyzed the 12,111 (77%) who reported at least immersing their body in the water to improve the accuracy of exposure classification based on cell counts. As part of a sensitivity analysis, more substantial exposures were considered, including those who reported swallowing water as a marker of extreme exposure (N= 5,615). Among participants with body immersion, the mean duration spent in the water was just over 2 hours. A quarter of all participants spent at least 3 hours in the water with the maximum time spent in the water at 8 hours.

Illness after beach visit

During the 10-12 day period between the beach visit and the follow-up telephone interview, respiratory illness was most commonly reported with an overall incidence of 7%. The incidence was 5% for GI illness, 5% for rash, 3% for eye irritation, and 2% for earache.

Water Quality

During the 26 study days, the median phytoplankton cell count was 712 cells/mL per day (range: 346 - 2,012 cells/mL). Of all groups identified, Bacillariophyta had the highest median

of 386 cells/mL per day. Cyanobacteria (excluding Picocyanophytes) had a median of 132 cells/mL per day. Picocyanophytes were only detected on 8 days but achieved a maximum count of 126,891 cells/mL. Dinophyta had a lower median of 37 cells/mL per day. Samples below the limit of detection (<0.2 cells/mL) were assigned a value of 0 for calculating phytoplankton distributions. Table 4 summarizes the phytoplankton distribution over the study period.

Other phytoplankton groups were detected on less than 8 study days at very low cell counts. These groups included Haptophyta (mean=8.6 cells/mL), Chrysophyta (mean=2.8 cells/mL), Rhodophyta (mean=0.7 cells/mL), Euglenophyta (mean=0.2 cells/mL), and Chlorophyta (mean=0.1 cells/mL). See Appendix 1 for a list of the genera and morphotypes that were identified, and their distribution, according to phytoplankton group. Concentrations of lyngbyatoxin-a and debromoaplysiatoxin were below the limit of detection of 1.0 ppb in every sample.

Enterococcus CFU and phytoplankton counts were not correlated (Spearman's $r=-0.14$, p -value=0.5). As previously reported by Wade et al. (2011), low to moderate levels of fecal indicator bacteria were detected at Boquerón Beach and the geometric means of the daily samples collected were all below the EPA criteria concentration of 35 CFU per 100 mL (Wade et al. 2011). Spearman's correlations between total phytoplankton counts and different environmental factors (e.g., wind speed and direction, air temperature, water temperature, turbidity) were all below 0.3 (data not shown).

Phytoplankton count and incident illness

Among beachgoers who reported body immersion, the highest category of total phytoplankton cell count ($\geq 75^{\text{th}}$ percentile) was associated with eye irritation (Adjusted Odds

Ratio (OR)=1.30; 95% confidence interval (CI): 1.01-1.66), rash (OR=1.27; 95% CI: 1.02-1.57), and earache (OR=1.25; 95% CI: 0.88-1.77) (Table 5). Cyanobacteria cell counts were associated with respiratory illness, eye irritation, rash, and earache. These associations, although not all statistically significant at $\alpha=0.05$, strengthened with increasing Cyanobacteria cell count categories (Figure 2). In particular, respiratory illness, rash, and earache all had associations that increased relatively monotonically with each Cyanobacteria cell count category. Respiratory illness also appeared to have the strongest association at the medium ($>25^{\text{th}}$ to $<75^{\text{th}}$ percentile) Cyanobacteria cell count category (OR= 1.30; 95% CI: 1.08, 1.56). Table 5 shows the associations between phytoplankton group cell count and incident illness among beachgoers who reported body immersion.

Among participants who reported body immersion in the water, Picocyanophytes were not associated with any subsequent illness (Table 5). However, among those who reported swallowing water (N=5,615), the presence of Picocyanophytes was significantly associated with earache (OR=1.62; 95% CI: 1.14-2.30), with earache being reported by 3.3% (N=56) of those exposed and 2.1% (N=83) of those unexposed. Similarly, among participants who reported swallowing water, the highest category of Cyanobacteria cell count was associated with earache (OR=1.75; 95% CI: 1.09-2.82), with earache being reported by 3.2% (N=52) of those exposed to the highest category and 1.8% (N=29) of those exposed to the lowest category.

As shown in Table 5, the medium category of Bacillariophyta was associated with respiratory illness (OR=1.28; 95% CI 1.07-1.54); however, the odds ratio did not increase with the highest category (OR=1.10; 95% CI: 0.89-1.35). Exposure to Dinophyta was not associated with any illness.

To assess potential effect measure modification by duration spent in water, we conducted stratified analyses by number of hours spent in the water (<1 hour, 1-<2 hrs, 2-<3 hrs, ≥ 3 hrs). We also restricted our analysis to other categories of water exposure (head immersion, swallowing water). Results were similar in stratified analyses, with the exception of a stronger earache association with Cyanobacteria and Picocyanophytes after restricting to participants who reported swallowing water (data not shown).

Discussion

We report the results of a prospective evaluation of the health effects associated with recreational water exposure to marine phytoplankton in the absence of a harmful algal bloom. Given the popularity of visiting beaches (Leeworthy et al. 2005) and the apparent increase in harmful algal blooms around the world (Sellner et al. 2003), we sought to better understand the effect of marine phytoplankton on human health. We found an association between total phytoplankton cell count and incident illness – specifically eye irritation and rash. These outcomes have also been associated with freshwater blooms (Billings 1981; Pilotto et al. 1997; Rapala et al. 2005; Walker et al. 2008).

Our study design established a temporal sequence between exposure and outcome. By having interviews at different time points, participants did not have to wait long to recall their experiences and their exposure response could not be influenced by any subsequent illness. The high participation rate (>75%) reduced the possibility of selection bias. After considering a range of potential confounders, we only adjusted for age (as a continuous variable) and sex in order to balance model parsimony and fit. Since our final model produced similar results to the full model (adjusting for age, sex, any other chronic illnesses, self-reported contact with algae,

Enterococcus count, and digging in sand), there did not appear to be a major bias due to confounding (data not shown).

Our study design allowed one adult to answer questions for all other household members at the beach. Although there was a possibility for responder bias or misinformation, there was only an average of 3.2 individuals per household and other household members often assisted with the questionnaire responses. A limitation to our self-reported outcome data was a lack of specific details for some of the illnesses. Therefore it is difficult to confirm the etiology of illness on the basis of the participant responses alone. For example, we could not necessarily distinguish among rashes as being associated with cnidarians, sea lice, cercariae, saltwater itself, or even something completely unrelated to the beach visit.

Cyanobacteria concentration was associated with all illnesses except GI illness; the odds of illness increased with cell count category. Our findings were consistent with reports of skin and eye irritations associated with *Lyngbya majuscula* blooms (Osborne et al. 2007; Osborne and Shaw 2008). Despite these similar illnesses, the *Lyngbya*-associated toxins, debromoaplysiatoxin and lyngbyatoxin-a, were below the limit of detection in all samples and *Lyngbya* only comprised 3% of total planktonic Cyanobacteria among samples (see Appendix 1). Debromoaplysiatoxin and lyngbyatoxin-a are photolabile and are also unlikely to persist in the water column (Moikeha et al. 1971). It is possible that people had contact with toxins or toxic material in the water or on the ocean floor as we did not sample the seabed or measure other cyanotoxins potentially associated with Cyanobacteria.

Unlike previous epidemiologic studies of freshwater cyanobacterial blooms (Levesque et al. 2014; Pilotto et al. 1997), GI illness was the only illness that did not appear to be associated with marine Cyanobacteria in the absence of blooms, even when we restricted the analysis to

participants who reported swallowing water. While the lack of association with GI illness could be due to low Cyanobacteria cell counts, health effects may also differ after exposure to communities of Cyanobacteria in fresh and marine waters. Maximum Cyanobacteria cell counts (excluding Picocyanophytes) were 1460.8 cells/mL. In epidemiologic studies conducted at freshwater sites, illnesses were associated with cyanobacterial counts >5,000 cells/mL (Pilotto et al. 1997), and at <20,000 cells/mL relative to no water contact (Levesque et al. 2014). More work is needed to define cyanobacteria concentrations safe for human health in marine waters.

We analyzed Picocyanophytes separately because of the different magnitude of cell counts. While most of the cyanobacteria literature describes marine picoplankton as non-toxic, there are some reports of toxic effects by homogenized *Synechococcus* and *Synechocystis* and their extracts (Martins et al. 2005; Martins et al. 2007; Walsh 2008). Microcystins have demonstrated adverse health effects (Codd et al. 1999; Falconer 1999; Giannuzzi et al. 2011) and a study of *Synechococcus* strains suggested the possibility that some marine picoplankton may be capable of synthesizing microcystins (Carmichael and Li 2006).

We observed an association between earache and Cyanobacteria among those who reported swallowing water. This is consistent with the positive but non-significant association we estimated among all those who immersed themselves in water. In the context of earache, we hypothesize that the stronger association, when restricted to those who swallowed water, may reflect more frequent head immersion and more intense exposure overall, rather than a direct consequence of swallowing water. Earache has been associated with swimming, especially when the head is immersed (Wade et al. 2013). The presence of any Picocyanophytes (versus none detected) was associated with earache among participants who reported swallowing water. To our knowledge, this association has not been previously reported.

Associations with specific outcomes varied among other phytoplankton groups. Bacillariophyta cell counts in the 25th to 75th percentile range, but not counts above the 75th percentile, were significantly associated with respiratory illness when compared with counts below the 25th percentile. To our knowledge, Bacillariophyta has not been associated with respiratory illness in previous studies. Previous studies of marine diatoms, such as those of the genus *Pseudo-nitzschia* that produce domoic acid, have focused on the adverse outcomes occurring after ingestion of contaminated shellfish rather than recreational water exposure (Van Dolah 2000).

Since phytoplankton cell counts were low, we cannot be confident that associations with health outcomes were a result of phytoplankton exposure alone or if phytoplankton were markers for other unmeasured causative factors, such as potentially pathogenic microbes or physical-chemical conditions associated with marine phytoplankton. Also, we are unable to rule out non-causal mechanisms related to chance or bias (e.g., uncontrolled confounding, selection bias, information bias). While phytoplankton may provide nutrients and substrate for microbial communities to survive, there is limited knowledge on the occurrence of phytoplankton-associated pathogens (Brettar et al. 2007; Maugeri et al. 2004).

We categorized cell count *a priori* as high ($\geq 75^{\text{th}}$ percentile), medium ($> 25^{\text{th}}$ to $< 75^{\text{th}}$ percentile), and low ($\leq 25^{\text{th}}$ percentile) because health guidelines for concentrations of marine phytoplankton have yet to be established. As a result, it is possible that the highest cell count category was actually below any level of potential adverse health effect. For example, although we did not find an association of illness with Dinophyta, maximum cell counts were only 105.9 cells/mL. In contrast, epidemiologic studies of the marine dinoflagellate, *K. brevis*, and

associated respiratory illness measured maximum cell counts ranging from 8,120 cell/mL (Backer et al. 2003) to 121,000 cells/mL (Backer et al. 2005).

We reported cell counts per milliliter of water so that our findings could be compared to previous studies and the WHO guidelines for freshwater exposures (Bartram and Chorus 1999). A limitation to our phytoplankton assessment was that we had no information on cell size to calculate biomass concentrations. Cell size can influence total phytoplankton exposure. For example, a few large cells of one species may contribute more to the overall biomass than many small cells of a different species (Hillebrand et al. 1999).

Lastly, our study participants spent much time in the water, with half of them spending at least two hours. It is possible that we observed associations between health outcomes and low phytoplankton counts, in the absence of active phytoplankton blooms, because participants spent so much time in the water. However, associations did not vary significantly when stratified by total time in the water (data not shown).

Our results offer insight into the potential health effects of marine phytoplankton in the absence of a harmful algal bloom. Although some associations could be due to chance or bias, most seem plausible based on the existing literature. The evaluation of health effects associated with recreational exposure to marine phytoplankton at sub-bloom concentrations warrants further investigation.

Conclusions

We found associations between recreational exposure to marine phytoplankton and subsequent reports of eye irritation, respiratory illness, earache and rash at a tropical beach in the absence of an algal bloom. In addition, we found that associations varied by phytoplankton group, with Cyanobacteria having the strongest associations with most of the outcomes assessed.

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TABLE 1. Definitions and exclusion criteria for outcomes that occurred during the 10-12 day period between the beach visit and follow-up telephone interview.

Outcome	Definition	Baseline conditions ^a excluded from analysis
Gastrointestinal illness	Any of following: diarrhea (3 or more loose stools in a 24-hour period); vomiting; nausea and stomach ache; nausea or stomach ache, and interference with regular activities	Gastrointestinal illness or vomiting
Rash	Rash or itchy skin	Rash
Respiratory illness	Any 2 of the following: sore throat, cough, runny nose, cold, or fever	Sore throat
Earache	Earache, ear infection, or runny ears	Earache
Eye irritation	Eye irritation or infection, watery eye	Eye infection

^a Baseline conditions occurred within 3 days before the beach visit.

TABLE 2. Characteristics of study population by level of water contact.

Characteristic	N (%) of all participants (n=15,726)	N (%) of participants reporting body immersion (n=12,111)
Sex		
Male	7052 (44.8)	5664 (46.8)
Female	8654 (55.0)	6431 (53.1)
Missing	20 (0.1)	16 (0.1)
Age category		
0-4	908 (5.8)	764 (6.3)
5-11	1791 (11.4)	1678 (13.9)
12-19	2272 (14.5)	1940 (16.0)
20-34	4407 (28.0)	3422 (28.3)
35-64	5594 (35.6)	3866 (31.9)
65+	540 (3.4)	284 (2.3)
Missing	214 (1.4)	157 (1.3)
Ethnicity		
Non-Hispanic	104 (0.7)	75 (0.6)
Hispanic	15609 (99.3)	12027 (99.3)
Missing	13 (0.1)	9 (0.1)
Visits to this beach each summer		
0-1	4592 (29.2)	3507 (29.0)
2-5	7533 (47.9)	5841 (48.2)
More than 5	3600 (22.9)	2762 (22.8)
Missing	1 (0)	1 (0)
Miles travelled to beach		
0-20	5161 (32.8)	3878 (32.0)
20-60	5597 (35.6)	4339 (35.8)
60-100	2795 (17.8)	2162 (17.9)
>100	1106 (7.0)	863 (7.1)
Missing	1067 (6.8)	869 (7.2)
Baseline health in the 3 days prior to beach visit		
Vomiting	130 (0.8)	97 (0.8)
Other gastrointestinal illness	309 (2.0)	232 (1.9)
Sore throat	1263 (8.0)	951 (7.9)
Rash	350 (2.2)	254 (2.1)
Eye irritation	153 (1.0)	98 (0.8)
Earache	224 (1.4)	164 (1.4)
Chronic illness		
Any (gastrointestinal, skin, respiratory)	3878 (24.7)	2992 (24.7)
Chronic GI illness	756 (4.8)	545 (4.5)
Chronic skin condition	548 (3.5)	401 (3.3)
Allergies	2010 (12.8)	1540 (12.7)
Asthma	1684 (10.7)	1352 (11.2)

TABLE 3. Activities reported on the beach exit interview.

Activity	N	(%)
All	15726	(100)
No water contact	2995	(19.0)
Total time spent in water		
<60 minutes	5547	(35.3)
60-<120 minutes	3445	(21.9)
120-<180 minutes	2835	(18.0)
180+ minutes	3892	(24.7)
Body immersion	12111	(77.0)
Head immersion	10074	(64.1)
Water in mouth	5615	(35.7)
Played with algae/seaweed	2499	(15.9)
Any contact with unknown animals	646	(4.1)
Dug in sand	3699	(23.5)

TABLE 4. Phytoplankton distribution over 26 days.

Phytoplankton	No. (%) of days present	Minimum (cells/mL)	Maximum (cells/mL)	Mean (cells/mL)	Std. Deviation (cells/mL)	25th percentile (cells/mL)	Median (cells/mL)	75th percentile (cells/mL)
All group/phyla combined	26 (100)	345.7	2011.7	788.9	381.3	581.6	711.6	783.4
Bacillariophyta	26 (100)	128.6	619.4	377.5	102.6	301.2	385.9	442.5
Cyanobacteria (excluding Picocyanophytes)	24 (92)	0	1460.8	254.4	379.4	36.7	131.6	237.4
Picocyanophytes	8 (31)	0	126891.2	22606.4	38020.8	0	0	45126.8
Dinophyta	26 (100)	0.2	105.9	38.9	28.3	23.5	36.6	54.6

Samples below the limit of detection (<0.2 cells/mL) were assigned a value of 0.

TABLE 5. Associations between phytoplankton cell counts and incident illness occurring during the 10-12 day period between the beach visit and follow-up telephone interview among beachgoers who reported body immersion in water.

Phytoplankton Group	Gastrointestinal illness (N=11,832)		Respiratory illness (N=11,160)		Eye irritation (N=12,013)		Rash (N=11,857)		Earache (N=11,947)	
	Cases (%)	OR (95% CI)	Cases (%)	OR (95% CI)	Cases (%)	OR (95% CI)	Cases (%)	OR (95% CI)	Cases (%)	OR (95% CI)
All groups combined (≤Q1)	174 (5.0%)	1.	227 (6.9%)	1.	114 (3.2%)	1.	156 (4.5%)	1.	57 (1.6%)	1.
All groups combined (>Q1 to <Q3)	211 (4.5%)	0.91 (0.74, 1.12)	315 (7.1%)	1.03 (0.86, 1.23)	144 (3.0%)	0.93 (0.72, 1.19)	199 (4.2%)	0.95 (0.77, 1.18)	96 (2.0%)	1.25 (0.90, 1.74)
All groups combined (≥Q3)	178 (4.9%)	1.00 (0.80, 1.24)	244 (7.1%)	1.02 (0.85, 1.24)	155 (4.2%)	1.30 (1.01, 1.66)	206 (5.6%)	1.27 (1.02, 1.57)	76 (2.1%)	1.25 (0.88, 1.77)
Bacillariophyta (≤Q1)	142 (4.4%)	1.	189 (6.1%)	1.	110 (3.3%)	1.	170 (5.2%)	1.	54 (1.6%)	1.
Bacillariophyta (>Q1 to <Q3)	281 (5.0%)	1.13 (0.92, 1.39)	405 (7.7%)	1.28 (1.07, 1.54)	223 (3.9%)	1.18 (0.94, 1.49)	279 (5.0%)	0.97 (0.80, 1.18)	118 (2.1%)	1.29 (0.93, 1.79)
Bacillariophyta (≥Q3)	140 (4.7%)	1.06 (0.83, 1.35)	192 (6.8%)	1.10 (0.89, 1.35)	80 (2.7%)	0.78 (0.58, 1.05)	112 (3.8%)	0.73 (0.57, 0.94)	57 (1.9%)	1.15 (0.79, 1.68)
Cyanobacteria (≤Q1)	173 (5.0%)	1.	187 (5.8%)	1.	114 (3.3%)	1.	144 (4.2%)	1.	55 (1.6%)	1.
Cyanobacteria (>Q1 to <Q3)	226 (4.4%)	0.87 (0.71, 1.06)	354 (7.4%)	1.30 (1.08, 1.56)	161 (3.1%)	0.96 (0.75, 1.22)	236 (4.6%)	1.12 (0.90, 1.39)	101 (2.0%)	1.25 (0.89, 1.74)
Cyanobacteria (≥Q3)	164 (5.0%)	0.98 (0.79, 1.22)	245 (7.8%)	1.37 (1.12, 1.67)	138 (4.1%)	1.25 (0.97, 1.62)	181 (5.5%)	1.32 (1.05, 1.66)	73 (2.2%)	1.35 (0.95, 1.93)
Picocyanophyte (none)	397 (4.8%)	1.	527 (6.7%)	1.	281 (3.3%)	1.	407 (4.9%)	1.	155 (1.8%)	1.
Picocyanophyte (any)	166 (4.7%)	0.96 (0.80, 1.16)	259 (7.8%)	1.13 (0.97, 1.32)	132 (3.7%)	1.10 (0.89, 1.36)	154 (4.3%)	0.90 (0.74, 1.09)	74 (2.1%)	1.11 (0.83, 1.47)

Dinophyta (≤Q1)	128 (4.5%)	1.	192 (7.2%)	1.	96 (3.3%)	1.	151 (5.3%)	1.	64 (2.2%)	1.
Dinophyta (>Q1 to <Q3)	275 (5.3%)	1.22 (0.98, 1.51)	358 (7.2%)	1.01 (0.84, 1.21)	185 (3.5%)	1.04 (0.81, 1.33)	243 (4.6%)	0.84 (0.68, 1.04)	87 (1.6%)	0.72 (0.52, 1.01)
Dinophyta (≥Q3)	160 (4.3%)	0.97 (0.76, 1.23)	236 (6.7%)	0.95 (0.78, 1.15)	132 (3.5%)	1.05 (0.80, 1.37)	167 (4.4%)	0.82 (0.65, 1.03)	78 (2.1%)	0.93 (0.67, 1.30)

Abbreviations: OR, Adjusted odds ratio; 95% CI, 95% confidence interval; Q1, 25th percentile; Q3, 75th percentile; N/A, not applicable
Models adjusted for age (as a continuous variable) and sex. Denominator for the case percentage is the total number in the exposure group.

Figure Legends

Figure 1. Water sampling sites at Boquerón Beach.

Figure 2. Associations between Cyanobacteria cell count and illness among beachgoers who reported body immersion in water. Models adjusted for age (as a continuous variable) and sex. Q1=25th percentile; Q3=75th percentile.

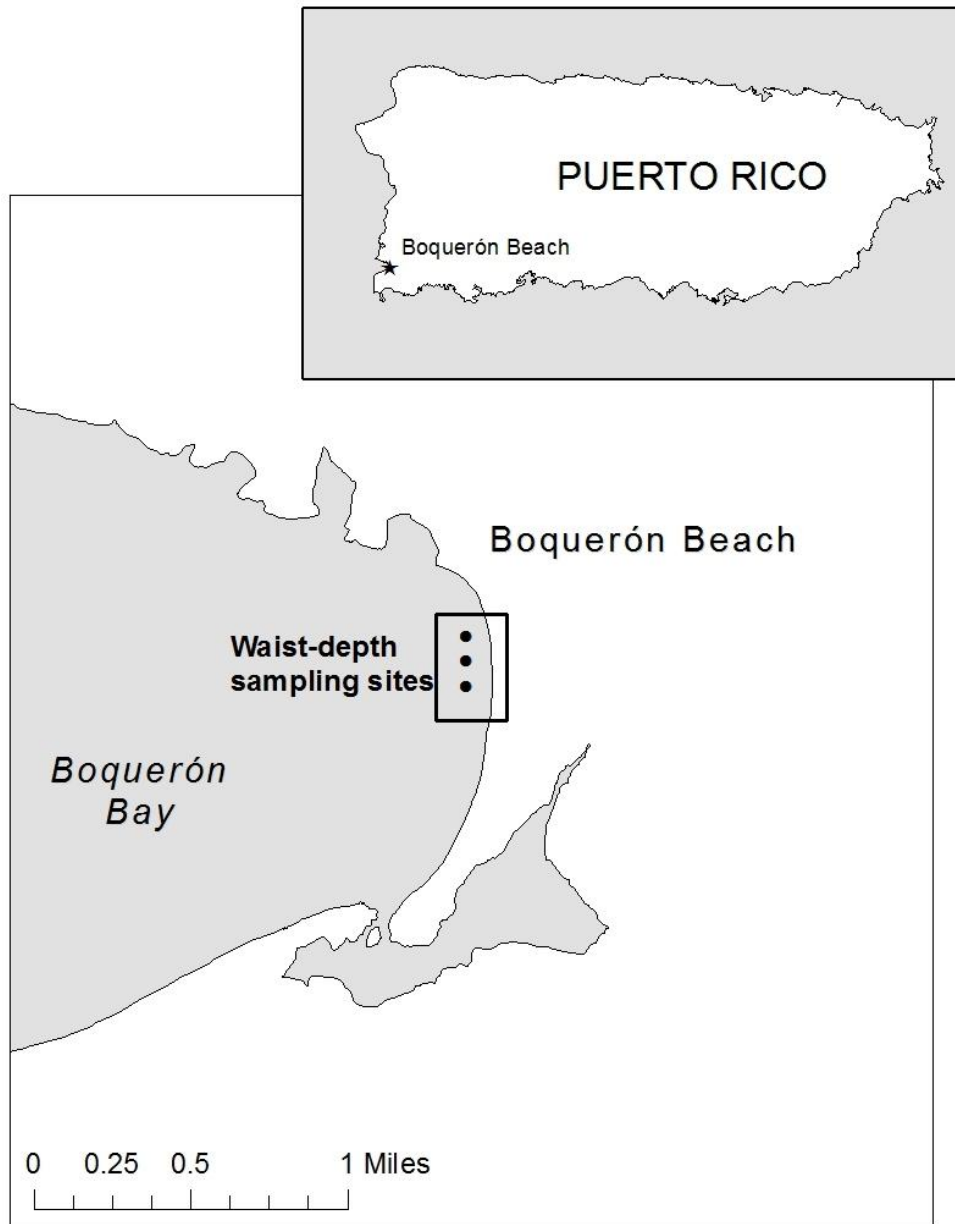


Figure 1. Water sampling sites at Boquerón Beach.

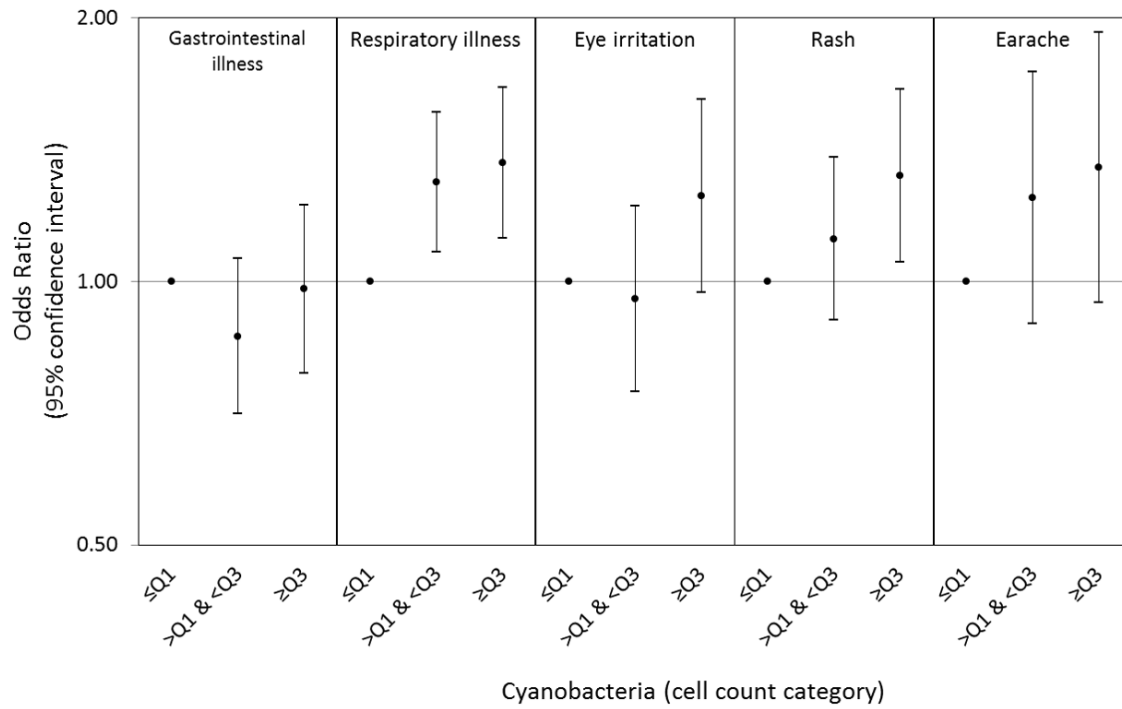


Figure 2. Associations between Cyanobacteria cell count and illness among beachgoers who reported body immersion in water. Models adjusted for age and sex. Q1=25th percentile; Q3=75th percentile.

Appendix 1. Genera/morphotypes by phytoplankton group (percentage within each group)

Bacillariophyta (69% of total phytoplankton)

Pennate Diatom (37); Nitzschia (12); Navicula (7); Licmophora (5); Bacillaria (4);
Cylindrotheca/Nitzschia (4); Amphiprora (4); Psammodictyon (4); Chaetoceros (3);
Centric Diatom (3); Diploneis (2); Amphora (2); Rhizosolenia (2);
Amphiprora/Plagiotropis (1); Actinocyclus/Coscinodiscus (1); Proboscia (1);
Skeletonema (1); Cocconeis (1); Gyrosigma/Pleurosigma (1); Actinoptychus (1);
Bacteriastrum (1); Diatom (1); Paralia (1); Achmannanthes (<1); Plagiotropis (<1);
Pseudonitzschia (<1); Triceratium (<1); Attheya (<1); Guinardia (<1); Gyrosigma (<1);
Odontella (<1); Peralia (<1); Tropidoneis (<1)

Dinophyta (13% of total phytoplankton)

Dinoflagellate (67); Gonyaulax (16); Ceratium (7); Prorocentrum (7); Amphidinium (1);
Dinophysis (1); Protoperidinium (1)

Cyanobacteria (9% of total phytoplankton)

Cyanophyte Filament (32); Pseudanabaena (26); Picocyanophyte (10); Synechococcus
(5); Synechocystis (5); Cyanophyte Cell Pair (5); Phormidium (4); Lyngbya (3);
Trichodesmium (3); Aphanothece (1); Johannesbaptistia (1); Komvophoron (1);
Cyanophyte Colony (1); Cyanophyte Unicell, Sphere 2.5-5um (1)

Miscellaneous (8% of total phytoplankton)

Unknown Flagellate (29); Unicell, Sphere 2.5-5um (26); Unicell, Oval 2.5-5um (14);
Unknown Unicell (11); Unicell, Oval 5-7.5um (10); Microflagellate (4); Unicell,
Oval/Rod 2.5-5um (3); Unicell, Sphere 5-7.5um (1); Unknown Filament (1)

Haptophyta (0.8% of total phytoplankton)

Haptophyte Flagellate (100)

Euglenophyta (0.6% of total phytoplankton)

Euglenophyte (80)
Euglena/Eutreptiella (20)

Chrysophyta (0.2% of total phytoplankton)

Chrysophyte Flagellate (100)

Rhodophyta (0.2% of total phytoplankton)

Rhodophyte Filament (100)

Chlorophyta (0.1% of total phytoplankton)

Chlorophyte Filament (100)